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13 Attachment of Bacterial Human Pathogens on Fruit and Vegetable Surfaces

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CONTENTS

13.1 INTRODUCTION

The microbiological safety of fresh and minimally processed fruits and vegetables has been questioned as a result of recent outbreaks of foodborne illness associated with unpasteurized juices, sprouts, melons, lettuce, berries and other commodities.

* Mention of a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of similar nature not mentioned.

The presence and survival of human pathogens in these commodities have been demonstrated. Fruits and vegetables are frequently in contact with soil, insects, animals, or humans during growing or harvesting [1] and in the processing plant. Thus, their surfaces are exposed to natural contaminants, and by the time they reach the packing house, most fresh produce retains populations of 10^4 to 10^6 microorganisms/g [1,2]. Many vegetables, including bean sprouts, cabbage, cucumber, potatoes, and radishes, have been found to be contaminated with *Listeria monocytogenes* [1,3–6]. This microorganism has been isolated from soil, sewage sludge, vegetation, and water [3,4] and therefore has the potential to contaminate produce surfaces. Despite several guides to the produce and fresh-cut industry on how to reduce microbial food safety hazards for fresh-cut fruits and vegetables [7,8], the incidence of salmonellosis is frequently reported. *Salmonella* is among the most frequently reported cause of foodborne outbreaks of gastroenteritis in the U.S. [9,10].

The ability of pathogenic and spoilage-causing bacteria to adhere to surfaces of fruits and vegetables continues to be a potential food safety problem of great concern to the produce industry. Surface structure and biochemical characteristics of bacteria and of a substratum, in this case fruits and vegetables, play a major role in how and where bacteria may attach [11].

13.2 OUTBREAKS OF FOODBORNE ILLNESS ASSOCIATED WITH PRODUCE

The number of documented outbreaks of human infections associated with the consumption of raw fruits and vegetables has increased in recent years. In the U.S. the number of reported produce-related outbreaks per year doubled between the period from 1973 to 1987 and 1988 to 1992 [10,12]. Five (1990,1991, 2000, 2001, 2002) multistate outbreaks of salmonellosis have been associated epidemiologically with cantaloupes. The first involved *Salmonella* Chester, which affected 245 individuals (two deaths) in 30 states. The second involved more than 400 laboratory-confirmed *Salmonella* Poona infections and occurred in 23 states and Canada [10]. The most recent (April/May 2002) outbreak was due to *Salmonella* Poona associated with 43 illnesses [13]. Other human pathogens including *E. coli* O157:H7 and *Shigella* are capable of growth on melon flesh [14,15]. The recent FDA survey of imported fresh produce reported an incidence of 5.3% positives for *Salmonella* and 2% for *Shigella* for 151 samples of cantaloupe. All contaminated melons originated in Mexico, Costa Rica and Guatemala [16]. In a survey of domestic fresh produce [17], of 115 samples of cantaloupes 2.6% were positive for *Salmonella* and 0.9% were positive for *Shigella*.

Among the greatest concerns with human pathogens on fresh fruits and vegetables are enteric pathogens (e.g., *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella*) that have the potential for growth prior to consumption or have a low infectious dose. More recently, outbreaks of salmonellosis have been linked to tomatoes, seed sprouts, cantaloupe, mamey, apple juice, and orange juice [18]. *Escherichia coli* O157:H7 infection has been associated with lettuce, sprouts, and apple juice, and enterotoxigenic *E. coli* has been linked to carrots (19,20). Documented

associations of shigellosis with lettuce, scallions, and parsley; cholera with strawberries; parasitic diseases with raspberries, basil, and apple cider; hepatitis A virus with lettuce, raspberries, and frozen strawberries; and Norwalk/Norwalk-like virus with melon, salad, and celery have been made [21,22]. A better understanding of bacterial adhesion to fruits and vegetables is needed for the development of more effective washing treatments to control microorganisms on fresh-cut produce.

13.3 SURFACES OF FRUITS AND VEGETABLES

13.3.1 Surface Characteristics

The surfaces of fruits and vegetables show a large diversity in structure and composition; the epidermis is covered by an epicuticular wax on aerial organs (leaves, stem, flowers, and fruits) or periderm on roots and tubers. Stomata, lenticels, broken trichomes, and scars from detached organs represent natural ways of entry for microorganisms. Cracks in the surface of vegetable and fruits may occur in certain growing conditions [23,24], and postharvest handling may cause injuries and bruising.

Unlike fruits with smooth surfaces, the outer surface (rind) of a cantaloupe presents a variety of surfaces to which a bacterium may bind. The epidermal cell surface is ruptured with a meshwork of raised tissue (the net). This net consists of lenticels and phellum (cork) cells. These cells have hydrophobic suberized walls to reduce water loss and protect against pathogen ingress. Also imparting a hydrophobic nature to the outer surface of cantaloupe is the cuticle composed of waxes and cutin that covers the epidermal cells [25]. Hydrophilic components of plant cell walls and middle lamella may also be exposed to bacterial invasion due to cuticular cracks and injuries to the epidermal surface.

13.3.2 Native Microflora of Fruits and Vegetables

There is great variation in the number and type of native microflora among fruits and vegetables due in part to their surface structures, chemical composition, type of fruits or vegetables, growth and harvesting condition, including processing treatments before, during, or after storage. It has been reported that 40 to 70% of the total flora of peas, snap beans, and corn consist of leuconostocs, streptococci, and corynebacteria, a Gram-positive rod [26]. Others are groups of bacteria that cause soft rot. Fruits contain less water and more carbohydrates and therefore would support growth of bacteria, yeast and mold. However, the pH of fruits is below the level that generally favors bacterial growth [27].

13.3.3 Types of Spoilage Microflora

Microorganisms responsible for postharvest diseases are not necessarily dominant on the surface of sound vegetables. On cabbage leaves *Botrytis cinerea* represented less than 0.1% of the total microflora [28], and on witloof chicory only 3% of the epiphytic isolates caused spoilage upon inoculation [29]. Some spoilage microorganisms may be specific to a few vegetable species, whereas others, such as *Botrytis*

TABLE 13.1
Resuscitation of Acid-Injured *Erwinia carotovora* subsp. *carotovora* (Ecc) Cells on Cut Surfaces of Cucumber and Apple Fruits

Incubation Time (h)	Plate Count (log cfu/membrane) on Cucumber			Plate Count (log cfu/membrane) on Apple		
	BHIA	VRBA	% Injury	BHIA	VRBA	% Injury
0	5.3[a] ± 0.2[b]	3.9 ± 0.1[b]	96[b]	4.9 ± 0.3[b]	3.8 ± 0.4[b]	93[b]
4	5.3 ± 0.1[b]	4.2 ± 0.2[c]	91[c]	4.7 ± 0.1[b]	3.7± 0.2[b]	94[b]
8	5.3 ± 0.3[b]	4.9 ± 0.3[d]	59[d]	4.3 ± 0.2[c]	3.0± 0.3[c]	95[b]
16	7.0 ± 0.4[c]	6.5 ± 0.2[e]	20[e]	4.5 ± 0.1[c]	2.9 ± 0.4[c]	97[b]

Note: Filter membranes containing acetic acid-treated Ecc (strain SR319 cells (0.3%, 6 min) were placed on cut surfaces of cucumber or apple fruits. The changes in the number of injured Ecc cells in the population were monitored after incubating the membranes containing acid-treated cells on either fruit for 4, 8, and 16 h. BHIA = brain heart infusion agar; VRBA = violet red bile agar. % Injury = (plate count on BHIA – plate count on VRBA)/plate count on BHIA] × 100%.

[a] The value represents the average of three experiments, two duplicates in each experiment ± standard deviation.

[b,c,d,e] Within a column, the numbers not followed by the same letter are significantly different ($P < 0.05$) by the Bonferroni LSD separation technique.

cinerea, *Sclerotinia* species, *Sclerotium rolfsii*, *Rhizopus stolonifer*, and soft rot bacteria, may attack a wide range of products. Many strains of *Alternaria alternata* and *Fusarium* spp. produce mycotoxins in decaying tomatoes [30] or potatoes [31,32]. However, a recent report concluded that vegetable products do not represent a significant hazard with regard to mycotoxins [33,34].

The relative importance of spoilage organisms for a given vegetable may differ in different countries and climates. *Mycocentrospora acerina* and *Phythophthora megasperma* were the main cause of spoilage of stored carrots in Normandy, France [35], whereas in England only *B. cinerea* and *Rhizoctonia carotae* were significant agents of spoilage [36]. In Denmark *R. carotae* was the major cause of postharvest decay [37]. On freshly harvested cabbage, *Alternaria tenuis* was the main cause of spoilage, *B. cinerea* prevailed on cabbage stored under air, and *Fusarium roseum* was an important cause of spoilage for cabbage stored under modified atmosphere [38]. At times injury may occur to bacterial cells on fruit and vegetable surfaces, probably due to adverse environmental conditions at the farm or during poststorage. Acid-injured *Erwinia carotovora* subsp. *carotovora* cells survived up to 16 h on cut surfaces of cucumber and apple fruits [39,40] (Table 13.1), and injured *Salmonella* Mbandaka or *Salmonella* Typhimurium also survived up to 16 h on fresh-cut apple disks (Table 13.2). Some microbial species do not cause spoilage of raw vegetables but may have an important impact on the quality of processed vegetables. Many postharvest disease agents colonize the mother plant, produce inoculum, and contaminate the vegetables before harvest [41]. This is the case with *Botrytis* spp., *Fusarium* spp., and *S. rolfsii* on various vegetable crops, *Phytophthora* spp. on

TABLE 13.2
Differential Responses of *Salmonella* Mbandaka and *S.* Typhimurium on Fresh-Cut Surfaces of Apple Disks to Water and Acetic Acid Treatment (2.4%, 5 min)

	Log Cells Killed (CFU/disk)[a] on the Disks Inoculated with:		Log (%) Cells Injured on the Disks Inoculated with:	
Washed with:	*S.* Mbandaka	*S.* Typhimurium	*S.* Mbandaka	*S.* Typhimurium
None	0.00[f]	0.00[f]	0.02 (4)[c f]	0.07 (14)[e]
Water	0.60[b e]	0.63[e]	0.03 (7)[ef]	0.08 (17)[e]
2.4% acetic acid	1.13[d]	1.41[d]	0.26 (45)[de]	0.29 (45)[d]
Water, then 2.4% acetic acid	1.17[d]	1.41[d]	0.32 (51)[d]	0.26 (45)[d]

[a] Initial cell numbers of *S. mbandaka* or *S. typhimurium* on the disk were in the range of 7.30 to 7.34 log cfu/disk.

[b] Values represent the average of three experiments, two duplication for each experiment.

[c] Numbers in parentheses represent the rate of injury in percentage; injury was determined based on the counts on XLT4.

[d,e,f] Within a column, the means followed by the same letter are not significantly different ($P < 0.05$) by the Bonferroni LSD separation technique.

solanaceous vegetables, *Alternaria* spp. on carrots, and soft rot erwinias on potatoes [42]. The plant or its immediate environment may also provide conditions for survival of microorganisms not commonly found in the field. For example, the soft rot bacterium *E. carotovora* survived for a longer period in the rhizosphere of crops than in the soil; pectolytic fluorescent pseudomonads were not found in soil [43] but were abundant in a field where carrots had suffered extensive soft rot [44]. *Listeria monocytogenes* is also suspected to survive better in the rhizosphere of plants than in nonrhizosphere soil [45]. Pathogenic bacteria [46–50] viruses [21,22,48,51] and helminths [53] declined on cultivated plants, but in some studies pathogens survived until harvest.

13.3.4 Sources of Bacterial Pathogen Contamination

It is very difficult to determine the primary source of contamination that leads to an outbreak, especially for produce. Determining the primary source of the pathogen will help in devising strategies and interventions to minimize risks of future outbreaks. For example, only 2 of 27 outbreaks during a particular time period of investigations of fresh produce were clearly identified to a point of contamination [54]. Bacterial pathogens may contaminate fruits and vegetables at any point throughout the production system. Potential preharvest sources of contamination include soil, feces, irrigation water, water used to apply fungicides and insecticides, dust, insects, inadequately composted manure, wild and domestic animals, and human handling [1]. *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* can be found in animal feces. Transmission of *E. coli* O157:H7 from manure-contaminated soil and irrigation water to lettuce plants and its migration throughout the plant were recently reported [55,56]. Evidence of an association of salmonellae

with stems and leaves of tomato plants grown hydroponically in inoculated solution has been presented [57]. Postharvest sources of contamination include feces, human handling, harvesting equipment, transport containers, wild and domestic animals, insects, dust, rinse water, ice, transport vehicles, and processing equipment [58].

13.4 ATTACHMENT OF HUMAN BACTERIAL PATHOGENS ON FRUITS AND VEGETABLES

The mechanism of attachment of bacterial cells to plant surfaces has been studied most extensively for plant pathogens and symbionts [59,60]. According to Fletcher [61], bacterial adhesion occurs in three steps: reversible adsorption, primary adhesion and colonization. During the reversible adsorption phase, the bacterium is at a distance of greater than 50 nm and is affected by van der Waal interactions with the substratum. This means that the bacteria can be easily washed off at this stage. At the primary adhesion stage, the distance between the bacteria and the substratum ranges from 10 to 20 nm and the type of force affecting adhesion is electrostatic unless the opposing surface has a net surface charge, then attractive forces will come into play. The colonization step is the final phase and biofilms may be formed. According to Buscher et al. [62], once bacteria overcome the water barrier and a separation distance of about less than 1.0 nm, additional adhesion interactions such as hydrogen bonding, cation bridging, and receptor-ligand interactions between bacteria and plant surfaces will occur. At this stage the bacteria are very difficult to remove. Flagella, fimbriae, outer membrane proteins and extracellular polysaccharides have all been implicated in attachment. Cellulose production and the presence of curli may allow for strong attachment of *Salmonella* to produce surfaces. Under natural conditions when contamination occurs in the field the production of cellulose and curli by *Salmonella* may allow for the bacterium to strongly bind to the plant surface and be highly resistant to removal by rain or by washing steps during processing. Attachment of bacterial human pathogens to fruits and vegetables has not been fully investigated.

Bacterial attachment to fruits with primarily smooth surfaces (apples, tomatoes, pears, and honeydew melons) involves less surface area than bacterial attachment to fruits with greater surface roughness. Bacterial attachment on the surface of apple fruits was greater in the vicinity of the calyx and stem, as compared to the remaining skin surface, with more than 94% recovery from the stem and calyx areas [63]. When a green pepper disk was inoculated, greater than 84% of the attached bacteria were on the injured surface, 15% on the inner skin, and less than 1% on the outer skin. Bacterial attachment was enhanced when the surfaces of apples or bell peppers were punctured or intentionally cut [14,63]. The adhesion of bacteria on the surface of cucumbers in wash water was less extensive at lower temperatures and shorter exposure times [64]. In this study the authors reported that various species of bacteria were adsorbed to cucumber surfaces in the following order: *Salmonella* Typhimurium > *Staphylococcus aureus* >*Lactobacillus plantarum* >*Listeria monocytogenes.* Cells were adsorbed at all temperatures tested (5, 15, 25, and 35°C) at levels that depended on incubation time, but the numbers of cells adsorbed were larger at higher

incubation temperatures. Levels of adhesion of bacteria to dewaxed cucumber were higher for *L. monocytogenes* and lower for *Salmonella* Typhimurium, *L. plantarum,* and *S. aureus* than were levels of adhesion to waxed cucumbers.

Some strains of *E. coli* O157:H7 are also known to produce fimbriae and bacterial exopolysaccharides [65,66], and these may function as plant surface adhesins. Studies using confocal scanning laser microscopy indicated that *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* can attach to intact plant surfaces, trichomes, and be present in substomatal chambers, but are found most often at wounded surfaces and within cuticular cracks [66–71]. Ukuku and Fett [11] reported higher initial adhesion of *E. coli* O157:H7 on melon surfaces compared to *Salmonella* and *L. monocytogenes*; however, *E. coli* O157:H7 were more easily removed than the other two pathogens by washing treatments. Barak et al. [13] reported that *Salmonella* binds more strongly on alfalfa sprouts than *E. coli* O157:H7.

13.4.1 Factors That Influence Bacterial Attachment

Most bacteria are readily suspended in aqueous medium because of the polar, hydrophilic moieties that abound on bacteria cell surfaces [72]. Hydrophilic sites consist of charged moities such as carboxyl, phosphate, amino, and guanidyl groups as well as neutral hydroxyl groups. Hydrophobic sites consist of lipids and lipopolysaccharides [73]. Bacterial surfaces are heterogeneous, with physicochemical properties determined primarily by teichoic acid (Gram-positive strains) or other polysaccharides (Gram-negative strains) along with proteinaceous appendages (fimbriae) [59,74,75]. The chemistry of teichoic acid or polysaccharides confers regions of hydrophobic or hydrophilic properties on bacterial surfaces, which aids in their attachment to surfaces. Bacterial attachment to surfaces is influenced not only by cell surface charge [76,77] and hydrophobicity [11,78–80] but also by the presence of particular surface appendages such as flagella and fimbriae as well as extracellular polysaccharides [81,82]. Flagella, fimbriae (pili), outer membrane proteins, and extracellular polysaccharide may influence bacterial attachment to plant surfaces [59]. Plant surfaces and microbes both have negative surface potential, which results in electrostatic repulsion between the two surfaces. Surface appendages such as pili already present on microbes prior to or induced by the presence of a plant surface or other favorable conditions are used to bridge the gap exerted by electrostatic repulsion [53]. It is difficult to predict the surface properties of bacterial human pathogens when the pathogens are first exposed to a plant surface because environmental conditions can significantly affect bacterial surface properties, including charge and hydrophobicity [66,78,83]. Specific interactions between complementary moieties such as bacterial carbohydrate polymers with plant lectins or fimbriae with plant carbohydrate-containing moieties may also play a role [84,85], especially in attachment to exposed plant cell wall materials and damaged tissues.

Recently, *Salmonella* was demonstrated to produce the extracellular carbohydrate polymer cellulose; this, along with curli (aggregative fimbriae), the two principle components of the extracellular matrix, is thought to be responsible for biofilm formation [86,87]. Interestingly, for the plant pathogen *Agrobacterium* and the plant

TABLE 13.3
Bacterial Hydrophobicity and Strength of Attachment to Cantaloupe Rind 24 Hours Postinoculation

Bacteria[a]	HIC[b]	S_R-value[c]
Salmonella spp.	0.484 + 0.118	0.934 + 0.010
Escherichia coli O157:H7	0.220 + 0.018	0.751 + 0.051
Listeria monocytogenes	0.281 + 0.063	0.816 + 0.036

[a] Inoculum for *Salmonella* spp. contained a cocktail of the following bacteria: *Salmonella* Stanley (3.43×10^8 CFU/mL), *Salmonella* Poona (2.65×10^8 CFU/mL), and *Salmonella* Saphra (2.46×10^8 CFU/mL).

Inoculum for *E. coli* cocktail consisted of ATCC 25922 (2.70×10^8 CFU/mL); 0157:H7-Odwala outbreak strain (2.38×10^8 CFU/mL); 0157:H7-Oklahoma outbreak strain (2.30×10^8 CFU/mL). *Listeria monocytogenes* inoculum consisted of 2.32×10^8 CFU/mL Scott A; 2.02×10^8 CFU/mL ATCC 15313; 2.30×10^8 CFU/mL CCR1-L-G; 2.32×10^8 CFU/mL H7778.

[b] HIC = hydrophobic interaction chromatography.
[c] S_R-value = strength of attachment.

symbiont *Rhizobium*, cellulose production plays an important role in the firm attachment of the bacteria to plants and in the formation of bacterial aggregates at the plant surface [53]. After initial attachment, *Agrobacterium* synthesizes cellulose fibrils that bind the bacteria very tightly to the host cell surface and to each other, and the bacterial cells can only be removed by digesting the bacterial or host cell wall [84]. Fibrils of unknown nature have been observed for *Pseudomonas putida* and *P. tolaasi* binding to the surface of *Agaricus bisporus* mycelium [89], *P. syringae* pv. *syringae* binding to apple tissues [90], and *Azospirillum brasilense* binding to tomato, cotton, and pepper roots [91]. Ukuku and Fett [11] reported that *Salmonella* had the highest S_R-value, followed by *L. monocytogenes* and then *E. coli* (Table 13.3). Higher S_R-values indicate stronger bacterial attachment to surface of melons, as indicated by the relative inability of washing treatments to detach the pathogen from the melons' surface using water. Also, surface hydrophobicity of *Salmonella* was higher than that of *E. coli* and *L. monocytogenes* (Table 13.3).

The overall cell surface charge for *Salmonella* varied among serovars; two isolates originating from cantaloupe (serovars Poona and Saphra) had higher cell surface charges than the serovar (Stanley) from alfalfa sprouts (Table 13.4). Strength of attachment on cantaloupe surfaces increased slightly for *E. coli* over 7 d of storage but decreased for *L. monocytogenes* [11]. There was no difference between cell surface charge for *E. coli* ATCC 25922 and *E. coli* O157:H7 strains (Table 13.5), and the cell surface charge for *L. monocytogenes* strains was very similar (Table 13.6). Among all the bacterial human pathogens tested, *Salmonella* had higher

TABLE 13.4
Relative Surface Charge for *Salmonella* spp. Determined by Electrostatic Interaction Chromatography

Serovar/strain	(–)	(+)	Source
Stanley HO558	21.48	4.10	Alfalfa sprout
Poona RM2350	33.71	1.82	Cantaloupe
Saphra 97A3312	50.00	6.08	Cantaloupe

Note: For experimental details see Ukuku, D. O. and Fett, W. F., *J. Food Prot.*, 65, 1093, 2002.

TABLE 13.5
Relative Surface Charge for *Escherichia coli* spp. Determined by Electrostatic Interaction Chromatography

Strain	(–)	(+)	Source
ATCC 25922	1.62	0.12	Type strain
O157:H7 (Odwala)	1.48	0.18	Apple juice
O157:H7 (Oklahoma)	1.50	0.16	Apple juice

Note: For experimental details see Ukuku, D. O. and Fett, W. F., *J. Food Prot.*, 65, 1093, 2002.

TABLE 13.6
Relative Surface Charge for *Listeria monocytogenes* Determined by Electrostatic Interaction Chromatography

Strain	(–)	(+)	Source
Scott A	38.06	0.40	Clinical isolate
CCR1-L-G	37.68	0.20	Food isolate
ATCC 151313	38.11	0.32	Type strain
H7778	37.47	0.50	Food isolate

Note: For experimental details see Ukuku, D. O. and Fett, W. F., *J. Food Prot.*, 65, 1093, 2002.

surface hydrophobicity and relative negative charges than *E. coli* and *Listeria monocytogenes*. However, *Listeria monocytogenes* had a significant relative cell surface charge than did *E. coli*. This heterogeneity may help explain the differences observed in bacterial hydrophobicity or cell surface charge in relation to their attachment to vegetable and fruit surfaces, especially the cantaloupe surface.

13.4.2 Factors Limiting Detachment of Microorganisms

Irregularities such as roughness, crevices, and pits have been shown to increase bacterial adherence by increasing cell attachment and reducing the ability to remove cells [92,93]. However, preventive mechanisms should be geared towards physical or chemical treatments to prevent bacterial transfer from the surfaces of the produce to the interior flesh. The effectiveness of chlorination of wash water in reducing the population of bacteria on produce is dependent on the interval between contamination and application of the washing treatment [70,94–96]. If bacterial attachment occurs more than 24 h prior to washing, detachment or inactivation using chlorine or hydrogen peroxide treatments was shown to be less effective, and the difference between the two treatments diminished. It is likely that the limited ability of washing to remove established bacterial populations from the surface of fresh produce is due in part to biofilm formation, microbial infiltration and internalization. At the retail level or at food establishments, produce is usually washed only using potable water, and the fresh-cut pieces may not always be prepared using clean and sanitized utensils. Thus, fresh-cut fruits and vegetables may not be adequately sanitized and protected from cross-contamination. However, because the time of contamination is not generally known and may precede washing by many days, more effective means of decontaminating produce are needed.

13.4.3 Biofilm Formation on Produce Surfaces

The ability of bacteria to form biofilms on food contact surfaces, which increases their resistance to cleaning and antimicrobial agents, is well known [15]. However, relatively little is known about biofilm formation on fruit and vegetable surfaces. Babic et al. [97] described biofilm-like structures associated with bacteria within spinach leaf tissue. Carmichael et al. [98] observed native bacterial biofilms on the surface of lettuce. Large differences in surface morphology and metabolic functions of different plant organs (e.g., fruits, flowers, leaves, and roots) provide a wide range of diverse ecological niches that could be selective for specific species or communities of microorganisms. Microbial growth on raw fruits and vegetables can result in the formation of biofilms by spoilage and nonspoilage microorganisms. These biofilms can provide a protective environment for pathogens and reduce the effectiveness of sanitizers and other inhibitory agents. A number of *Pseudomonas* species associated with plants can produce exopolysaccharides characteristic of biofilms [74,99]. Human pathogens including *Campylobacter jejuni*,
E. coli O157:H7, *L. monocytogenes* and *Salmonella* Typhimurium also are able to form biofilms on inert surfaces [15,100].

13.5 BACTERIAL DEGRADATION OF FRUIT AND VEGETABLE SURFACES

So far there is no reported evidence that suggests direct degradation of produce by human bacterial pathogens; rather, they may coexist with the spoilage organisms associated with the produce. Antagonistic relationships between pseudomonads and *Listeria monocytogenes* on potato slices have been reported [101]. *Salmonella* attached in greater numbers and survived washing with sanitizers to a greater extent on cut surfaces of pepper disks, compared to natural external or internal surfaces. Similarly, *Salmonella* attachment and survival during washing were greater in the calyx and stem areas and on cut surfaces of apples, compared to the unbroken skin surface [63].

Also, respiration, transpiration and enzymatic activity of living tissue after harvest can cause fruit and vegetable deterioration. Consumer evaluation of processed vegetables and fruits is based on the absence of discoloration, resulting from enzymatic browning of cut surfaces, and the yellowing of green vegetables [101–104]. The surfaces of fruits and vegetables are covered with epiticular wax and cuticle that function as a hydrophobic barrier to water and gas exchange, and the amount of this cuticle and epiticular wax vary from species to species; therefore, the postharvest enzymatic activity varies. The enzymes of particular interest are the pectolytic enzymes, which gradually cause the ripening of the fruits, making them softer, and chlorophyllase, which catalyzes the cleavage of phytol from chlorophyll to form chlorophyllides, which leads to the formation of pheophorbides. The browning or the darkening of fruits and vegetables is a significant problem, and several research efforts are currently underway to prevent these actions [102,105]. The surface matrix of fruits and vegetables is made up of cellulose, polyuronic acids, proteins, and phenolic compounds and waxes [105]. Ethylene promotes many changes in fruits and vegetables. The decrease of ethylene leads to the loss of green color in green peppers.

13.5.1 Prevention of Microbial Contamination and Degradation of Produce

There are no clear-cut answers as to how bacterial attachment to produce surfaces can be avoided or eliminated. Fruits and vegetables are frequently in contact with soil, insects, animals, or humans during growing or harvesting [106]. A major factor limiting the efficacy of conventional sanitizing treatments for apples and other commodities is the inaccessibility of attached bacteria. Several studies have demonstrated the presence of enteric bacteria, including Enterobacteriaceae and Pseudomonadaceae, within fresh tomatoes and cucumbers [43,107]. An evaluation of mature apples for the presence of *Erwinia amylovora* (cause of fire blight) could not detect this organism but did reveal the presence of internalized Enterobacteriaceae in about 5% of the samples examined [108]. Internalization of *E. coli* O157:H7 has been reported in lettuce [109,110] and radish sprouts [19,111].

The interventions aimed at reducing microbial spoilage on produce surfaces should be applied at preharvest, during harvesting, and postharvest. At preharvest,

intervention applications should consider cultural practices such as crop rotation, pruning of produce and destruction of crop debris. During harvesting, emphasis should be on hygiene (i.e., hygienic condition of the environment, the harvesters, and containers used for harvesting and transporting the produce to the storage facility). At postharvest, intervention technologies may include physical or chemical treatments geared towards reducing surface contaminants. Fumigation of fruits and vegetables during storage will reduce the potential for decay [112–114]. The efficacy of sanitizing treatments in decontaminating fruits and vegetables has been investigated [11,70,71,115–124], and population reductions of 2.6 to 3.8 $\log_{10}$ CFU/g have been reported for *Salmonella* and *E. coli* 0157:H7 [70,71,122–124]. However, some studies have shown that substantially smaller reductions are obtained when the targeted bacteria have been on the fruit surfaces for than a few days [11,70,71]. Limited bactericidal action (1 to 3 log reductions) of chlorine on produce, including whole melons, has been reported [2,11,20,96,125–128]. Depending upon the fruit or vegetable and whether it is whole or cut, up to 200 to 300 ppm chlorine is usually recommended as a sanitizer in wash water [20,129]. The lack of an effective antimicrobial treatment at any step from planting to consumption means that pathogens introduced at any point may be present on the final food product. Washing and rinsing some types of fruits and vegetables prolong shelf life by reducing the number of microorganisms on their surfaces. However, only a portion of pathogenic microorganisms may be removed with this simple treatment. Use of a disinfectant can enhance efficiency of removal up to 100-fold, but chemical treatments administered to whole and cut produce typically will not reduce populations of pathogens by more than 2 to 3 $\log_{10}$ CFU/g [129].

Pathogens also vary in their sensitivity to sanitizers. For example, *L. monocytogenes* is generally more resistant to chlorine than are *Salmonella* and *E. coli* O157:H7 [111,129,130]. The general lack of efficacy of sanitizers on raw fruits and vegetables can be attributed, in part, to their inaccessibility to locations within structures and tissues that harbor pathogens. Pathogenic bacteria are able to infiltrate cracks, crevices, and intercellular spaces of seeds and produce. Infiltration is dependent on temperature, time, and pressure and occurs when the water pressure on the produce surface overcomes internal gas pressure and the hydrophobic nature of the surface of the produce [18,62]. Infiltration may also be enhanced by the presence of surfactants and when the temperature of the fruit or vegetable is higher than the temperature of contaminated wash water. The protective mechanism of these sites is not well understood but the concept that hydrophobicity of microbial cells aids in their protection by inhibiting penetration of the disinfectants has been proposed. Recently, Ukuku et al. [131] reported reduced microbial populations of fresh-cut melon prepared from whole melon treated with hot water. In this study they concluded that decontamination of whole cantaloupes designated for fresh-cut processing with hot water could have major advantages over the use of sanitizers, including a significant reduction or elimination of vegetative cells of pathogenic bacteria on melon surfaces, thus reducing the probability of potential transfer of pathogenic bacteria from the rind to the interior tissue during cutting. Although application of sanitizer and heat treatment has been used to kill *Salmonella* Stanley inoculated on

alfalfa seeds [132] and vegetables [133], this type treatment was able to reduce the population of native microflora and inoculated *Salmonella* on whole melon surfaces without compromising the quality of the fresh-cut pieces [132,133]. Alternatively, lactic acid bacteria can be used to improve the microbial safety of minimally processed fruits and vegetables [42,134–138]. As with infiltration, preharvest internalization of human pathogens within fruits and vegetables would greatly limit the efficacy of washing as a means of decontamination.

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